Similar Influence of Stabilized Alkaline and Neutral Sodium Hypochlorite Solutions on the Fracture Resistance of Root Canal–treated Bovine Teeth

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Abstract

Introduction: Stabilizing sodium hypochlorite (NaOCl) at an alkaline pH is proposed to increase solution stability and tissue dissolution ability; however, a reduction on the flexural strength of dentin discs has been found to be a side effect. This study sought to determine whether a stabilized alkaline NaOCl reduces the fracture resistance of root canal–treated bovine teeth after root canal preparation compared with a neutral solution counterpart. **Methods:** The 4 anterior incisors were removed from 20 mandibular bovine jaws, and each 1 was randomly assigned to 1 of 4 groups (20 teeth each). Teeth were prepared with a sequence of 6 K-type files. The following experimental groups received a different irrigation regimen: G1: distilled water (negative control), G2: 5% NaOCl at a pH of 7.2, and G3: 5% NaOCl at a pH of 12.8; in the positive control group (G4), teeth remained untreated. The time of contact and volume of solution were carefully standardized. After bone and periodontal ligament simulation, teeth were subjected to a fracture resistance test. **Results:** A significant difference was observed among the 4 groups tested (analysis of variance, $P < .05$). The 5% NaOCl groups (G2 and G3) presented significantly lower resistance to fracture than the control (G1 and G4) (Tukey test, $P < .05$). Both NaOCl solutions similarly reduced the fracture resistance at approximately 30% (Tukey test, $P > .05$). No differences were observed between positive and negative control groups (Tukey test, $P > .05$). **Conclusions:** Stabilized alkaline and neutral NaOCl solutions similarly reduced the fracture resistance of root canal–treated bovine teeth by about 30%. *(J Endod 2014;40:1600–1603)*

Key Words

Alkaline NaOCl, bovine teeth, fracture resistance of endodontically treated teeth

The use of sodium hypochlorite (NaOCl) solutions largely remains the mainstream approach for root canal disinfection because of the unique tissue proteolysis capacity and microbial suppression by NaOCl (1, 2). These essential properties of NaOCl solutions are predominantly influenced by the amount of available chlorine (3, 4). In NaOCl solutions, chlorine can take different chemical forms depending on the solution's pH. At an alkaline pH, the predominant form is hypochlorite (ClO^-), whereas at a neutral pH the hypochlorous acid form predominates (5). The latter is considered to be more bactericidal than hypochlorite (6); thus, it seems appropriate to adjust the pH of NaOCl to a neutral level with the purpose of increasing its antimicrobial effectiveness (7). However, at a pH of 7.5, NaOCl was found to be unstable, which causes a severe reduction in its shelf life (7, 8), preventing the neutralized solution from being marketed on a regular basis. In addition, the drop in hypochlorite ion renders neutralized NaOCl more cytotoxic (8) and less effective in dissolving organic tissue (9) because the cleaning effectiveness of NaOCl solutions is related to the presence of ClO^- (8, 10, 11). Therefore, all NaOCl solutions available for clinical use are alkaline.

Albeit alkaline, NaOCl solutions rapidly show a drop in pH when active chlorine is consumed during interaction with tissues and microorganisms (3, 5, 9), which, in turn, result in a severe decline in the solution's ability to dissolve organic tissue (9). Recently, the effect of adding an alkali with the aim of maintaining the stability of the solution and preserving its capacity to dissolve organic tissue has been investigated (9). Some available household bleach and dental-marketed NaOCl solutions are currently adding alkali to provide stabilization and to increase the shelf life of NaOCl (12), while also claiming a superior proteolytic effect.

Unquestionably, tissue proteolysis encompasses a pivotal feature of NaOCl because either vital or necrotic tissue remnants may became a potential source for root canal reinfection in cases of incomplete canal disinfection or leakage. Nonetheless, the proteolytic action of NaOCl also negatively impacts on dentin, causing the depletion of its components of organic nature (13). This highly undesired NaOCl side effect irreversibly changes the dentin framework, causing a dry weight reduction by 14% (14). Therefore, the occurrence of physical and mechanical changes in dentin disks, such as microcrack formation and the reduction in flexural strength, microhardness, and modulus of elasticity after NaOCl use is not a surprise (14–23).

Following a cause-effect rationale, the increase in the proteolytic effect triggered by a stabilized alkaline NaOCl solution (8–10) may intensify the side effect on the organic scaffold of dentin, ultimately leading to the undesired end result of root weakening

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(9, 13, 23). In fact, stabilized alkaline NaOCl induced a severe decrease in the elastic modulus and flexure strength of dentin discs compared with a nonstabilized counterpart (9). Because weakened roots may significantly impact tooth survival, the effect of stabilized alkaline NaOCl over fracture resistance should be investigated.

We aimed to evaluate whether the use of a stabilized alkaline NaOCl solution influences the strength of root canal–treated bovine teeth compared with a neutral NaOCl solution counterpart. The null hypothesis is that NaOCl solutions at 2 different pH levels do not influence the fracture resistance of bovine teeth.

Materials and Methods

Sample Size Calculation

An analysis of variance (ANOVA) fixed-effects model (F family, G*Power 3.1.1 for Windows, Heinrich-Heine-Universität Düsseldorf, Germany) was used to set the ideal sample size. Based on a pilot study, the effect size was determined at 0.58; an alpha-type error = 0.05 and power $\beta = 0.95$ were input. The results indicated a minimum total sample size of 56 teeth and a critical F of 2.79 for the ANOVA evaluation.

Sample Selection and Preparation

Twenty mandibular bovine jaws from cattles similar in age were selected to provide the 4 anterior incisors. Animals were slaughtered for feeding purposes, and the jaws were donated to this study. With the aim of providing anatomic matching among the groups, each selected incisor was randomly assigned to 1 of 4 groups, resulting in 20 teeth per group and a total sample size of 80 teeth. For each jaw, the 4 incisors were measured at a level 8 mm from the apex to ensure roots display a 8–10 mm diameter. Root diameters lower or lager than the established parameters results in the exclusion of the cow. After extraction, teeth were stored in saline until use.

All teeth were cross-sectioned at levels of 8 mm coronally and 12 mm apically to the cement/enamel junction by means of a low-speed saw (VC-50 Precision Diamond Saw; Leco, Miami, FL) under copious water cooling resulting in samples with lengths of 20 mm. The pulpal tissue was removed with Hedströen files (Dentsply Maillefer, Ballaigues, Switzerland).

Preparation and Characterization of Irrigating Solutions

Freshly prepared technical-grade NaOCl solutions were obtained from a pharmacy (Special Farma, São Luis, Brazil). A 2-mol/L NaOH solution was mixed with a standard 10% NaOCl solution to obtain an NaOH-stabilized alkaline 5% NaOCl solution (9). The neutral solution was acquired by mixing a standard 10% NaOCl solution with 1% sodium bicarbonate ($NaHCO_3$) (24). The available chlorine of the solutions was certified using a standard iodine/thiosulfate titration method immediately before and after the experiments (20 days later). Before and after the experiment, the pH of the solutions was verified using a calibrated pH electrode (Model 6.0210.100; Metrohm, Herisau, Switzerland). The pH of NaOH-stabilized NaOCl was also determined after 40 minutes of contact with a bovine root canal to confirm that the stabilization was effective (9). All root canal preparations were performed in a controlled room temperature.

Root Canal Instrumentation and Irrigation

The apical portion of each tooth was sealed off with wax, preventing any irrigation liquid from being extruded from the large apical opening created after apical sectioning. Groups G1–G3 received different irrigation regimes as follows: G1: distilled water (negative control), G2: 5% sodium hypochlorite with a pH of 7.2, and G3: 5% NaOCl with a pH of 12.8. In the positive control group (G4), teeth remained untreated.

To ensure irrigated groups (G1–G3) received the same volume of irrigation, root canals were instrumented using a sequence of 6 hand K-files (Dentsply Maillefer), which were selected after determining the first instrument to bind at 1 mm from the apical opening. After each hand file, 5 mL irrigation solution was delivered into the root canal using a 27-G endodontic needle (NaviTip; Ultradent Products, South Jordan, UT) reaching 3 mm from the apex. A constant rate of 1 mL/min was achieved using a VATEA peristaltic pump (ReDent Nova, Ra'anana, Israel). After irrigation, the root canals remained filled with the solution, and the subsequent instrument was used to prepare the root canal using a step-back technique. Each instrument was used for 2 minutes. Considering the number of instruments used (6), the total period that root canal dentin remained in contact with the solutions was 26 minutes. The extruded solution was aspirated adjacently to the coronal opening to make sure that any solution was drawn off the external root surface. All teeth received a final irrigation with 10 mL distilled water for 5 minutes, removing any solution remnants from the root canal. Furthermore, root canals were dried with paper points and stored at 37°C with 100% humidity until the strength tests were performed.

Simulation of the Periodontal Support Apparatus

Teeth were firstly immersed in melted wax (Horus; Herpo Produtos Dentários, Petrópolis, RJ, Brazil) up to 2.0 mm below the cementoenamel junction to create a 0.2- to 0.3-mm-thick wax layer covering the root. Furthermore, a polystyrene resin (Cristal, Piracicaba, Brazil) was used to embed the roots in polyvinyl chloride cylinders (a 21-mm diameter and 25-mm high). After the resin was set, the teeth were withdrawn from the polyvinyl chloride cylinders, and the wax removed from root surface and resin cylinder "sockets" using a warm water flush for 2 seconds. A polyether impression material (Impregum Soft; 3 M/ESPE, Seefeld, Germany) was delivered to the cylinder hole using a syringe. The samples were immediately reinserted into the respective cylinder socket, and any excess of impression material was removed, finally resulting in a simulated periodontal ligament of 0.2–0.3 mm (25).

Fracture Strength Test

All specimens were subjected to a compressive load at a crosshead speed of 0.5 mm/min by means of a servo-hydraulic universal testing machine (EMIC DL2000; EMIC Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, Brazil) until fracture. The specimen was fixed to an apparatus that allowed a 45° angle formation with the EMIC loading tip, simulating a traumatic shock on the middle third of the crowns from a buccal-lingual direction. The ultimate load required to fracture the specimens was recorded in newtons.

Statistical Analysis

Raw data adhesion to Gaussian distribution and homogeneity of the variance were studied a priori (Shapiro-Wilk and Levene tests). Because both assumptions were confirmed ($P > .05$), 1-way ANOVA followed by the Tukey Honest Significant Difference post hoc test were selected to verify the effect of the solution in the fracture strength of bovine teeth. The α-type error was set to 0.05.

Results

Table 1 displays the mean and standard deviations of the tested groups. One-way ANOVA indicated a significant difference between the groups ($P < .05$). The negative control group (distilled water) behaved similarly to positive controls because no significant change